Pergamon

Journal of Insect Physiology 49 (2003) 677–683

Journal of Insect Physiology

www.elsevier.com/locate/jinsphys

Ecdysone agonist halofenozide affects corpora allata and reproductive physiology of the Formosan subterranean termite, *Coptotermes formosanus*

Ashok K. Raina [a,*], Yong Ihl Park [a], Zuzana Hruska [b]

[a] *USDA, ARS, Formosan Subterranean Termite Research Unit, 1100 Robert E. Lee Blvd., New Orleans, LA, 70179, USA*
[b] *USDA, ARS, Cotton Fiber Quality Research Unit, 1100 Robert E. Lee Blvd., New Orleans, LA, 70179, USA*

Received 2 January 2003; received in revised form 17 March 2003; accepted 18 March 2003

Abstract

Following a short swarming flight, winged adults of the Formosan subterranean termite, *Coptotermes formosanus* lose their wings and form tandem pairs. These dealates or primary reproductives then form incipient colonies. Topical application of 5 μg of the non-steroidal ecdysone agonist RH-0345 (halofenozide) in 0.1 μl DMSO to the primary reproductives during the 2000 season resulted in significant reduction in the number of eggs laid. There was however complete recovery of the treated females. Apparently the non-treated partner removed the treatment chemical while grooming indicating oral activity. In 2001 both topical application as well as feeding methods were tried. Significant effects were observed only in the topical treatment group, perhaps because of inconsistency in feeding. In this group, total progeny, the number of ovarioles in ovaries and the size of the female's corpora allata (CA) were all significantly reduced. Ultrastructure of the CA of treated females showed extensive vacuolation near the surface of the gland. The experiment was repeated in 2002 using both topical application and an improved oral feeding method. Whereas there was apparent recovery in the topically treated group after 90 days, the oral treatment was more persistent in its effect perhaps due to a higher amount of halofenozide consumed during feeding.
Published by Elsevier Science Ltd.

Keywords: Ecdysteroid agonist; Halofenozide; RH-0345; Formosan subterranean termite; Corpora allata

1. Introduction

The Formosan subterranean termite, *Coptotermes formosanus*, introduced into the United States around the middle of the last century, has become a serious urban pest in Louisiana and several other southeastern states, causing damage to both houses and live trees (Woodson et al., 2001). Because of the environmental concerns, many of the conventional pesticides used for termite control have been taken out of the market. Among the newer chemicals is the group of chitin synthesis inhibitors which are used in baits for colony elimination (Raina et al., 2001). However, there continues to be a need to identify new environmentally safe technologies to control this menacing pest.

Growth and development in insects is mainly regulated by the molting hormone and juvenile hormone (JH), whereas JH regulates reproductive maturation in the adult stage. In insects, prothoracic glands are the main source of ecdysone and, JH is produced in the corpora allata (CA). A number of non-steroidal ecdysone agonists or bisacylhydrazines have been synthesized as novel pest control agents (Dhadialla et al., 1998). Symptoms caused by bisacylhydrazines are generally similar to those expected from excess ecdysteroids. In addition they are metabolically stable and show persistence in the insect body (Dhadialla et al., 1998). Tebufenozide (RH-5992) and methoxyfenozide (RH-2485), two of the bisacylhydrazines, are primarily active against lepidopteran species, causing precocious metamorphosis and death (Retnakaran et al., 1997; Smagghe et al., 2000, 2001; also review by Dhadialla et al., 1998). Halofenozide

* Corresponding author. Tel.: +1-504-286-4290; fax: +1-504-286-4235.
E-mail address: araina@srrc.ars.usda.gov (A.K. Raina).

0022-1910/03/$ - see front matter Published by Elsevier Science Ltd.
doi:10.1016/S0022-1910(03)00069-6

(RH-0345), on the other hand, has shown activity against coleopteran species (Soltani et al., 1998, 1999; Cowles et al., 1999; Farinós et al., 1999; Smagghe et al., 1999; Kunkel et al., 2001). The effect on ovarian development was the primary focus of most of these latter studies. An interesting property of the bisacylhydrazines is that they exhibited both topical and oral activity.

The Formosan subterranean termite like many termite species produce huge swarms of winged adults that disperse and form new colonies. In Louisiana, the alates of this species swarm for about six weeks from April to June. After a short flight, followed by loss of wings, the dealate pairs form new or incipient colonies. In order to target these primary reproductives, we investigated the effect of halofenozide on their reproductive physiology. The experiments were conducted during the swarming seasons of 2000–2002.

2. Materials and methods

2.1. Test insects and chemicals

Swarming alates of the Formosan subterranean termite were collected in black-light traps set in New Orleans, LA, during April and May. After dealation, the insects were sexed and individual pairs held in 30 ml plastic cups with snap-on lids. Technical grade halofenozide was provided by T. S. Dhadialla of Rohm and Haas, Norristown, PA (now Dow AgroSciences).

2.2. Experiments

During the 2000 season, 5 μg halofenozide in 0.1 μl DMSO was topically applied to the abdominal tergites. Four treatments with 30 pairs per treatment were set up as follows: 1) female treated, 2) male treated, 3) both female and male treated, and 4) control (both sexes treated with DMSO). After treatment, the pairs were placed singly in a 13 mm hole in agar-sawdust mixture poured into 50 × 9 mm Falcon tight-fitting petri dishes (Raina et al., 2003). Mortality and oviposition were recorded every third day for 30 days. After 30 days, five pairs from each treatment were dissected and the status of their ovaries/testes and CA determined. Based on the results (females showed the effect when males were topically treated, and not vice versa), two possibilities became apparent; one that halofenozide was orally active in female termites and second, that most of the topically applied material was removed by the untreated mate while grooming. Consequently, during the 2001 season four treatments with halofenozide were set up: 1) Filter paper (20 × 5 mm), 2) Agar-sawdust disk (3 mm thick, 7 mm dia), 3) Diet matrix (Rojas and Morales-Ramos, 2001) 12 mg, all treated with 70 μg in 10 μl acetone, and 4) Topical application to both female and male at 5 μg in 0.1 μl DMSO. Each treatment had its own control with 20 pairs in each treatment and control group. Observations on mortality and total progeny were recorded after 30 days. Number of ovarioles, size of testes and CA were determined from five randomly selected pairs from each treatment and control group. CA from treated and control females were also processed for electron microscopy. During the 2002 season, only two treatments were set up: 1) both females and males were topically treated with 5 μg halofenozide in 0.1 μl DMSO, and individuals held separately for 2 h after treatment before pairing, 2) A 10 mg piece of Kimwipes® EX-L tissue was treated with 100 μg halofenozide in 25 μl acetone. After evaporating the solvent, the tissue was wetted with 25 μl distilled water and provided to a dealate pair in a 30 ml plastic cup with snap-on cover. After 72 h, tissue pieces were visually scored for consumption and, the pairs that had consumed at least 30% of the tissue were transferred to the diet petri dishes. Observations on mortality and progeny were recorded at 30, 60 and 90 days. After 90 days, randomly selected pairs were dissected to examine ovaries and testes. Also, CA were dissected from females that had either recovered or not recovered from the treatment (based on number of progeny) and prepared for electron microscopy.

2.3. Electron microscopy

CA together with corpora cardiaca and a piece of the aorta were dissected from halofenozide treated and control females, 30 and 90 days after treatment, during the 2001 and 2002 seasons respectively. From the 2002 study, CA from the treated group were divided into two sub groups; females that appeared to have recovered from the treatment and those that did not. The tissues were dissected in PBS (0.137 M NaCl, 0.0027 Kcl in 0.01 M phosphate buffer, pH 7.4) and fixed in 3% glutaraldehyde in 0.05 M phosphate buffer for 48 h at room temperature. After fixation, the samples were washed several times with 0.05 M phosphate buffer and, post-fixed in 2% osmium tetraoxide in 0.05 M phosphate buffer for 2 h. After 2–3 washes the tissues were dehydrated in a graded ethanol series. Tissues were infiltrated with a series of low-viscosity medium (Spurr, 1969) in acetone, culminating with two changes in 100% resin. Blocks were cured at 60 °C for 24 h, trimmed and sectioned on an ultramicrotome using a diamond knife. Thin sections were placed on copper grids and stained with saturated uranyl acetate and 0.4% lead citrate. Sections were viewed with a Philips CM-120 transmission electron microscope.

2.4. Statistics

The 2000 data was analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test

for treatment vs control means. The 2001and 2002 data were analyzed using unpaired two-tailed t-test.

3. Results

3.1. Results of year 2000 test

Results from topical application of halofenozide to primary reproductives are presented in Fig. 1. Two of the treatments, only male treated and both female and male treated, had significant effect on fecundity (ANOVA, $F = 30.22$, $P = 0.0001$, followed by Dunnett's multiple comparison test, $P < 0.01$). The fact that the group in which females were treated did not show any significant reduction in egg laying was puzzling until it became obvious that the untreated individual in a pair actively removed the chemical by allogrooming. This observation indicated that halofenozide was orally active in the Formosan termite and, presented the possibility of application with a food source. Preliminary observations indicated that halofenozide affected the ovaries and CA, both of these being small in treated females compared to control females. Egg hatch was slightly delayed but there was no effect on percent hatch. Moreover, the effects were temporary and the treated females recovered with the start of the second reproductive cycle, or about three months following treatment.

3.2. Results of the 2001 test

Mortality among both control and treated groups in the agar-sawdust fed primary reproductives was high (>90% died within 30 days). Lowest mortality and no apparent effect was observed with diet matrix, probably because primary reproductives consumed very little of this material. Consequently data from these two treatments is not presented. With filter paper and topical application, mortality was especially high in the treated groups (Table 1). Total progeny numbers were significantly lower in feeding treatment ($t = 4.404$, $P = 0.0003$) as well as in topical treatment ($t = 8.586$, $P = 0.0001$) as compared to control. In addition, the number of ovarioles per ovary was significantly lower ($t = 4.886$, $P = 0.0001$) and the size of CA significantly smaller ($t = 10.55$, $P = 0.0001$) in females topically treated with halofenozide. The treatments did not affect either the testes or the CA in the males. At the ultrastructural level, the CA of control females appeared normal with well defined cells, their cytoplasm containing large number of mitochondria and microtubules (Fig. 2A). In comparison, CA of the treated females in the topical treatment group, had large number of vacuoles just below the gland surface (Fig. 2B,C). These vacuoles often contained remnants of membrane like structures (Fig. 2B). Also cytoplasm in the peripheral cells in CA of treated females contained high density of ribosomes, and dense bodies but no microtubules.

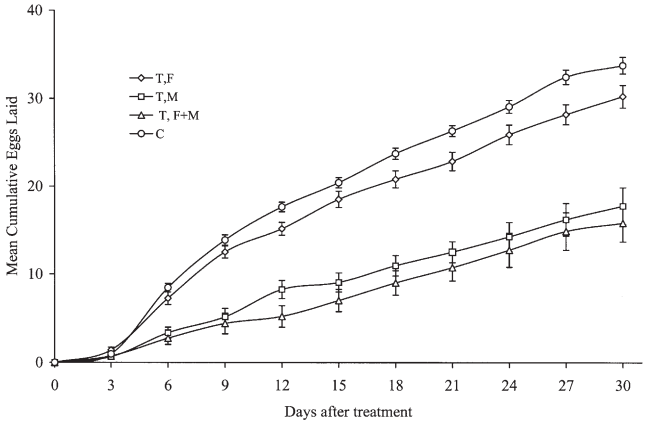

Fig. 1. Effect of topical application of 5 μg halofenozide in 0.1 μl DMSO on oviposition in the primary reproductive of *C. formosanus*. C = control ($N = 15$), TF = treated female ($N = 10$), TM = treated male ($N = 12$), TF + M = both male and female treated ($N = 9$). Values are mean ± SE.

3.3. Results of the 2002 test

Because of the low consumption of various test substrates during the 2001 test, we tested Kimwipes and found that feeding on this tissue was both extensive and consistent. Post-feeding mortality was between 30–50% irrespective of treatment. Based on visual scores, a pair of primary reproductives consumed from 30 to 80% of the tissue in 72 h. Total progeny after 30 days was significantly lower in both topical ($t = 13.32$, $P < 0.0001$) and oral ($t = 7.621$, $P < 0.0001$) treatment groups compared to their respective controls (Table 2). The progeny showed a slight decline at 60 days, even in the control groups, which is normal. The differences in progeny numbers between treated and control groups were significant. Females treated topically showed some recovery at 90 days whereas the orally fed group, as a whole, did not. However, based on individual progeny numbers, the latter group could be divided into two subgroups. One group designated as recovered had a mean progeny of 28.9 ± 2.9 ($N = 9$) and the second group or non-recovered had a mean progeny of 1.5 ± 1.1 ($N = 6$). Examination of both sexes in each group revealed that whereas, the ovaries and testes looked normal in the recovered group, these were almost degenerate in the non-recovered group. Further, females in the non-recovered group had no sperm in the spermatheca. The CA, particularly in the females, were also very small (0.0114±0.002 mm^2) compared to 0.024 ± 0.002 mm^2 in the recovered group.

Ultrastructure of CA from females of the two subgroups revealed that while the CA in the recovered group had attained normalcy the CA from the non-recovered

Table 1
Effect of feeding and topical application of halofenozide on mortality, progeny numbers, gonad development and size of corpora allata of primary reproductives of *Coptotermes formosanus*[d]

	Feeding (filter paper)		Topical application	
	Control	Treatment	Control	Treatment
Mortality (%)[a]	30.0	70.0	25.0	60.0
Total progeny[b]	36.2±4.3 (14)	25.7±6.1 (6)∗	27.1±3.3 (15)	12.2±5.0 (8)∗∗
Ovarioles/ovary	10.4±1.3	11.6±1.8	9.7±1.6	6.8±1.0∗∗
Testes (μm)[c]	466±21.2	448±39.1	350±14.1	338±17.5
CA-female (mm^2)[c]	0.030±0.003	0.031±0.004	0.026±0.002	0.012±0.002∗∗
CA-male (mm^2)	0.032±0.003	0.029±0.006	0.023±0.002	0.021±0.005

[a] N = 20 pairs each for control and treatment in each group.
[b] Progeny consisting of eggs and larvae were counted after 30 days of treatment, mean ± S.D. (N).
[c] Each testis measured at its broadest point, CA size = length × maximum width, $N = 5$.
[d] ∗,∗∗ Indicate significance in paired comparisons by two-tailed *t*-test between control and treated within each application method.

females had developed a convoluted outer surface reflecting the shrinkage in the gland (Fig. 2D). The vacuoles that were close to the surface of the gland, 30 days after treatment (Fig. 2C), had apparently coalesced to form large empty spaces in non-recovered females 90 days post-treatment.

4. Discussion

In insects ecdysteroids regulate many developmental and physiological processes especially growth and metamorphosis (Koolman, 1990). Following the discovery of bisacylhydrazines as non-steroidal ecdysone agonists (Wing et al., 1988), their potential in pest management has gained considerable attention (Oberlander et al., 1995). Whereas, two of these compounds, tebufenozide and methoxyphenozide are specifically active against Lepidoptera, another member, halofenozide is shown to be active against a number of coleopteran species (Dhadialla et al., 1998). Almost all studies with bisacylhydrazines have shown that they cause precocious metamorphosis when applied to immature stages of an insect. Their affect on adults is less clear.

In our first experiment with primary reproductives of the Formosan subterranean termite, we used topical application of halofenozide to either females, or males or both. Initially it was puzzling to see that fecundity was adversely affected only when males or both sexes were treated, until we realized that the material applied to a male was 'licked' by the female while grooming, an activity that is common and vital for the survival of termites. As an example, Rosengaus et al. (2000) reported that infection in the dealates of dampwood termite, *Zootermopsis angusticollis*, exposed to the fungus *Metarhizium anisopilae* depended on dose, as at lower dose susceptibility was decreased through allogrooming. Although oral activity of bisacylhydrazines is well documented, this is the first report of its activity in a social insect and through allogrooming. We also showed that halofenozide affected fecundity in the Formosan subterranean termite.

In tests conducted in 2001, we used 70 μg halofenozide on three types of substrates with the assumption that each primary reproductive will consume at least 10% of the material. There was no noticeable consumption in case of agar-sawdust and diet matrix and none of the parameters recorded showed any difference between control and treated groups. Among the other two treatments, only topical application caused significant reduction in progeny as had been noticed during the previous year. There was no difference in hatching between eggs laid by control and treated females. The number of ovarioles in each ovary was also significantly lower as was the size of female CA. There was no difference in testes and CA between control and treated males. Farinós et al. (1999) reported that topical application of 20 μg halofenozide to adult Colorado potato beetle, *Leptinotarsa decemlineata*, caused rapid cessation of oviposition due to degeneration of ovaries, detrimental oocyte growth and reduction in yolk protein synthesis. The effect was dose dependent, and at 10 μg, the cumulative number of eggs was reduced by 58% at 21 days. Using [^{14}C] halofenozide, Farinós et al. (1999) reported that while radioactivity in the integument of treated females decreased from 70% after one day to 13% after seven days, in the reproductive system it increased from 16 to 72% during the same period indicating selective affinity for the compound. Soltani et al. (1999) reported no effect on ovarian proteins and no evident effect on fine structure of follicular cells and oocytes when 5 μg halofenozide was applied to 0 or 2 day old adult females of *Tenebrio molitor*. However, a higher dose of 10 μg caused slight effect, particularly reducing the thickness of follicular epithelium, one of the sites of ecdysteroid synthesis. Similarly, in the ground beetle, *Harpalus pennsylvanicus*, treatment with halofenozide by topical application, or feeding method caused no apparent

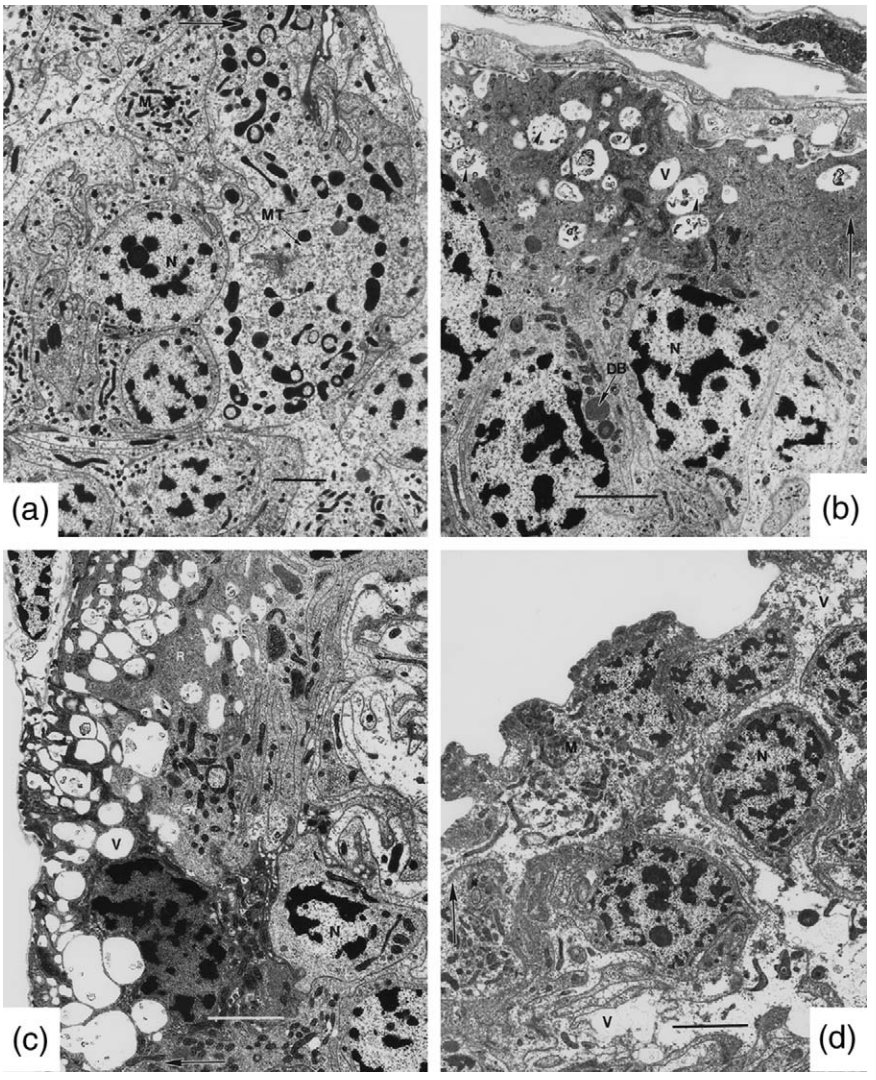

Fig. 2. Electron micrographs of the corpora allata (CA) from females of *C. formosanus*. (A) CA of a control female showing normal gland structure. (B, C) CA of a female 30 days following topical application with 5 μg halofenozide, showing extensive vacuolation near the surface of the gland. The vacuoles appear to contain remnants of membrane like structures (arrow heads) in B. (D) CA of a non-recovered female 90 days after orally feeding halofenozide. The gland surface shows convolutions. Arrow near the margin of each electron micrograph points to the outer surface of the gland. Abbreviations: DB, dense bodies; M, mitochondria; MT, microtubules; N, nucleus; R, ribosomes; V, vacuoles. Scale bars 2.0 μm (A, B, D), 2.5 μm (C).

Table 2
Effect of topical application and feeding halofenozide on the size of progeny in primary reproductives of *Coptotermes formosanus*

Treatments	Total progeny in days after treatment		
	30	60	90
Topical/control	37.4±1.1 (15)[a]	24.3±1.4 (13)	33.9±2.4 (12)
Topical/treated	16.8±1.1 (15)∗∗∗	15.4±2.2 (14)∗∗	25.8±2.8 (10)∗
Oral/control	34.0±1.3 (14)	27.9±1.6 (13)	34.9±2.2 (12)
Oral/treated	20.5±1.2 (20)∗∗∗	18.2±2.2 (17)∗∗	17.9±3.9 (15)∗∗

[a] Mean progeny ± SEM, numbers in parentheses indicate the pairs of primary reproductives. Control and treated means for a given day compared by unpaired *t*-test; $P < 0.0001$ (∗∗∗), $P < 0.05$ (∗∗,∗).

adverse effects (Kunkel et al., 2001). They further stated that females fed halofenozide-treated food, one time or continuously for 30 days, did not affect the viability of the eggs laid by treated females.

Perhaps most significant was the correlation between atrophy of ovaries and the small size of CA in topically treated females. At the ultrastructural level, the CA of treated females had a large number of vacuoles appear near the outer surface of the gland. The vacuoles appeared to contain remnants of membrane like structures. While the nuclei looked normal, the glial processes formed tight convolutions perhaps due to loss of cytoplasmic content of the cells. The 2002 results indicated that with topical application of 5 μg halofenozide, the progeny was significantly lower in the treated group, 30, 60 and 90 days after treatment, although at 90 days there was a clear trend of recovery. For the oral treatment, considering the amount of tissue paper consumed, each individual received 15–40 μg of halofenozide. Once again there was significant reduction in the progeny after 30 and 60 days. The discrepancy observed in the results from oral feeding between 2001 and 2002 was perhaps due to greater intake of the chemical during the latter year. Based on the progeny, at the end of 90 days, the treated group was divided into two subgroups; those that appeared to have recovered and those that did not. Examination of reproductive organs and CA of females in both subgroups revealed that ovaries in the recovered females resembled those in the control females. The CA too showed recovery with very few vacuoles present near the surface of the gland. Compared to this, females in the non-recovered group had degenerate ovaries, no sperm in the spermatheca and very small CA. At the ultrastructural level the CA showed further degeneration with the gland surface becoming convoluted. It is assumed that recovery from halofenozide treatment was dose dependent. Unnithan et al. (1977) reported inhibition of egg maturation and degeneration of the CA of *Oncopeltus fasciatus*, as a result of treatment with precocene, an anti-JH compound (Bowers et al., 1976). However, the degeneration of CA in the case of *O. fasciatus* was irreversible. Contrary to our results, Muszynska-Pytel et al. (1992) reported that in *Galleria mellonella*, application of the ecdysone mimic RH 5849 caused increased allatotropic activity resulting in supernumerary larval molts. This is the first report on the effect of an ecdysone agonist on the reproductive biology of a social insect with emphasis on the novel action on CA.

Acknowledgements

We thank Christopher Florane for technical assistance, Bruce Ingber for help in electron microscopy and Drs. Nan-Yao Su and Arthur Retnakaran for critically reviewing the manuscript.

References

Bowers, W.S., Ohta, T., Cleere, J.S., Marsella, P.A., 1976. Discovery of insect anti-juvenile hormones in plants. Science 193, 542–547.

Cowles, R.S., Alm, S.R., Villani, M.G., 1999. Selective toxicity of halofenozide to exotic white grubs (Coleoptera: Scarabaeidae). Journal of Economic Entomology 92, 427–434.

Dhadialla, T.S., Carlson, G.R., Le, D.T., 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Annual Review of Entomology 43, 545–569.

Farinós, G.P., Smagghe, G., Tirry, L., Castañera, P., 1999. Action and pharmacokinetics of a novel insect growth regulator, halofenozide, in adult beetles of *Aubeonymus mariaefranciscae* and *Leptinotarsa decemlineata*. Archives of Insect Biochemistry and Physiology 41, 201–213.

Koolman, J., 1990. Ecdysteroids. Zoological Science 7, 563–580.

Kunkel, B.A., Held, D.W., Potter, D.A., 2001. Lethal and sublethal effects of bendiocarb, halofenozide and imidacloprid on *Harpalus pennsylvanicus* (Coleptera: Carabidae) following different modes of exposure in turfgrass. Journal of Economic Entomology 94, 60–67.

Muszyńska-Pytel, M., Mikołajczyk, P., Pszczółkowski, M.A., Cymborowski, B., 1992. Juvenilizing effect of ecdysone mimic RH 5849 in *Galleria mellonella* larvae. Experientia 48, 1013–1017.

Oberlander, H., Silhacek, D.L., Porcheron, P., 1995. Non-steroidal ecdysteroid agonists: tools for the study of hormonal action. Archives of Insect Biochemistry and Physiology 28, 209–223.

Raina, A., Woodson, W., Lax, A., 2001. Current and future management strategies for subterranean termites. In: Muraleedharan, D., Nair, K.S.S., Mohandas, N., Tandon, P.L., Palaniswami, M.S., Jacob, M. (Eds.), Advances in Entomology, pp. 29–36.

Raina, A.K., Park, Y.I., Florane, C., 2003. Behavior and reproductive biology of the primary reproductives of the Formosan subterranean termite. Sociobiology 41, 37–48.

Retnakaran, A., Smith, L.F.R., Tomkins, W.L., Primavera, M., Palli, S.R., Payne, N., 1997. Effect of RH-5992, a non-steroidal ecdysone agonist, on the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae): laboratory, greehouse and ground spray trials. Canadian Entomologist 129, 871–885.

Rojas, M.G., Morales-Ramos, J.A., 2001. Bait matrix for delivery of chitin synthesis inhibitors to the Formosan subterranean termite. Journal of Economic Entomology 94, 506–510.

Rosengaus, R.B., Traniello, J.F.A., Lefebvre, M.L., Carlock, D.M., 2000. The social transmission of disease between adult male and female reproductives of the dampwood termite *Zootermopsis angusticollis*. Ethology Ecology and Evolution 12, 419–433.

Smagghe, G., Vinuela, E., van Limbergen, H., Budia, F., Tirry, T., 1999. Nonsteroidal moulting hormone agonists: effects on protein synthesis and cuticle formation in Colorado potato beetle larvae. Entomologia Experimentalis et Applicata 93, 1–8.

Smagghe, G., Carton, B., Heirman, A., Tirry, L., 2000. Toxicity of four dibenzoylhydrazine correlates with evagination-induction in cotton leafworm. Pesticide Biochemistry and Physiology 68, 49–58.

Smagghe, G., Carton, B., Decombel, L., Tirry, L., 2001. Significance of absorption, oxidation, and binding to toxicity of four ecdysone agonists in multi-resistant cotton leafworm. Archives of Insect Biochemistry and Physiology 46, 17–139.

Soltani, N., Smagghe, G., Soltani-Mazouni, N., 1998. Evaluation of the ecdysteroid agonist RH-0345 on the hormonal production by integumental explants and ovaries in mealworms. Mededelingen Faculteit Landbouwwetenschappen, Universitiet Gent 63, 547–554.

Soltani, N., Amrani, L., Soltani-Mazouni, N., Smagghe, G., 1999. Evaluation of the ecdysteroid agonist RH-0345 in mealworms:analysis of ovarian proteins and structure of follicle. Mededelingen Faculteit Landbouwwetenschappen, Universitiet Gent 64, 279–285.

Spurr, A.R., 1969. Low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastrutural Research 26, 31.
Unnithan, G.C., Nair, K.K., Bowers, W.S., 1977. Precocene-induced degeneration of the corpus allatum of adult females of the bug *Oncopeltus fasciatus*. Journal of Insect Physiology 23, 1081–1094.
Wing, K.D., Slawecki, R.A., Carlson, G.R., 1988. RH-5849, a nonsteroidal ecdysone agonist: Effects on larval Lepidoptera. Science 241, 470–472.
Woodson, W.D., Wiltz, B.A., Lax, A.R., 2001. Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. Sociobiology 37, 661–671.